Final Report for Pyronaridine_INV-054926-2 Project

Project	Pyronaridine_INV-054926-2
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Distribution

Irvin Zhou, Claude Mercier, Pyronaridine (INV-054926-2) project team, BMGF

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1. Introduction and Acknowledgements

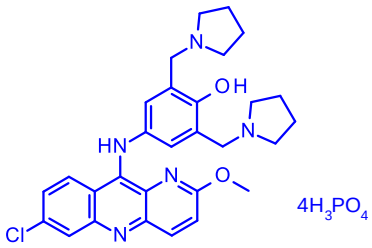
Pyronaridine tetraphosphate and Artesunate form the active ingredients of the anti-malaria treatment Pyramax.

The Bill & Melinda Gates Foundation (BMGF) asked for a partner to develop a cost-efficient and robust process to produce Pyronaridine tetraphosphate at a commercial scale. The strategy of BMGF is to sponsor a development and initial scale up program and to transfer the obtained process to an established API producer.

PHT tech Started the Phase Ⅰ work from May 2023 based on the initial study by Professor Lipshutz team from UC Santa Barbara, which was completed in December 2023. During Phase Ⅰ, three different routes **(Convergent route named as route 1, Linear route named as route 2 and modified convergent route named as route 3, see page 36)** were successfully developed. The study showed that impurity profile of API and costs were different for these 3 routes. And route 3 was defined as the best one among these three routes after the comparison on the raw material cost, operation cost and E factor.

Consequently, Phase Ⅱ work was proposed based on the study in Phase Ⅰ to do the further improvement on the process and to address the unsolved issues in Phase Ⅰ study. Phase Ⅱ work started from Feb 2024 and was completed at the end of May 2024.

This report combines the Phase Ⅰ and Phase Ⅱ work.



TGF-001
(PND-$4H_3PO_4$)
CAS 76748-86-2

With the completion of this project, we would like to express our heartfelt thanks to Prof. Bruce H. Lipshutz, professor at the University of California, Santa Barbara, Dr. Claude Mercier, the CTO of PHT International and BMGF expert team for their supporting throughout this project. Their extensive knowledge and expertise are fundamental in ensuring the success of this project.
We also would like to give our sincerely thanks to our colleagues, especially to Mr. Rack Dong and Mr. Harry Shi who are responsible for Process Development, Mr. Alain Cai who is for Analytical Development, for their innovative contributions and hard work in this project.

2. Executive summary

PHT have designed and completed a comparative study of three synthetic routes for pyronaridine phosphate. These three synthetic routes can meet the quality requirements of the Chinese Pharmacopoeia. But route 3 has the highest purity and lowest cost.

PHT have provided 3 choices for purification of API to meet the requirements of BGMF.

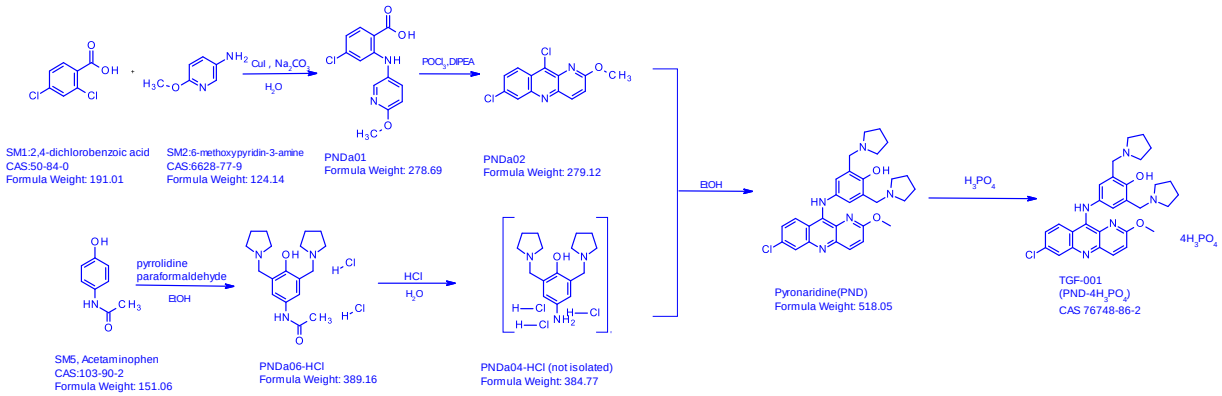
PHT have developed a cost-effective, environmentally friendly, and reliable process for scaling up the production of pyronaridine phosphate. This process can meet the continuously changing requirement.

PHT also developed a process for to make 5A2MP (key raw material). The overall yield of 3 steps was 56%.

PHT also completed a use test of 5A2MP from 3 different suppliers.

Impurity control in the API (TGF-001) is successful, however, the application of purification procedure of water/EtOH (additional 1 eq. H_3PO_4) to water/acetone purification directly is not suitable (OOS of HPLC assay). The reason maybe acetone cannot wash the additional H3PO4 as EtOH does.

Due to the late and rush request of the latest specification (HPLC purity and crystal form), the modified purification process gave the OOS material. It should be further investigated.



Scheme 1. Synthesis of TGF-001 by route 3

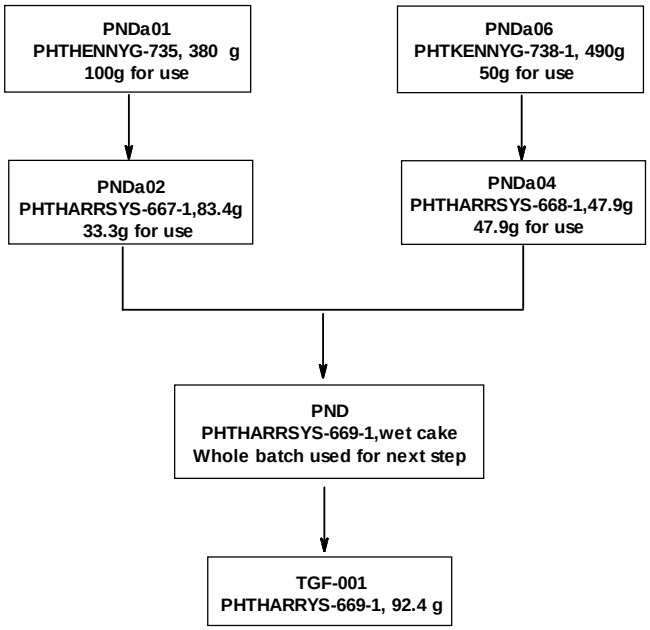
Abbreviation	Description
ADS	Analytical datasheet
MeCN	Acetonitrile
aq.	Aqueous
CuI	Copper(I) iodide
CP	Chinese Pharmacopoeia
DOE	Design Of Experiment
DIPEA	N, N-Diisopropylethylamine
eq.	Equivalent (s)
EA	Ethyl acetate
EtOH	Ethanol
h	Hour (s)
g	Gram (s)
HPLC	High performance liquid chromatography
HCl	Hydrogen chloride
H_3PO_4	Phosphoric acid
H_2O	Water
IPA	propan-2-ol
IPC	In process control
kg	kilogram (s)
L	Liter (s)
LOD	Loss on dry
Max.	Maximum
MT	More than
MeOH	Methanol
mL	Milliliter (s)
N/A	Not available
No.	Number
NMT	Not more than
Na_2CO_3	Sodium carbonate
NH_4OH	Ammonium hydroxide
NaOH	Sodium hydroxide
N_2	Nitrogen
N.D.	Not detected
$POCl_3$	phosphorus oxychloride
pH	Potential of hydrogen
ppm	Phases per million
RRT	Relative retention time
Res.	Residue
SM	Starting material
Spec.	Specification
Temp.	Temperature
THF	Tetrahydrofuran
U.I.	Unknown impurity
V	Volume (s)
v/w	Volume/ weight
w/w	Weight/weight
°C	Celsius degree

3. Result and Conclusion

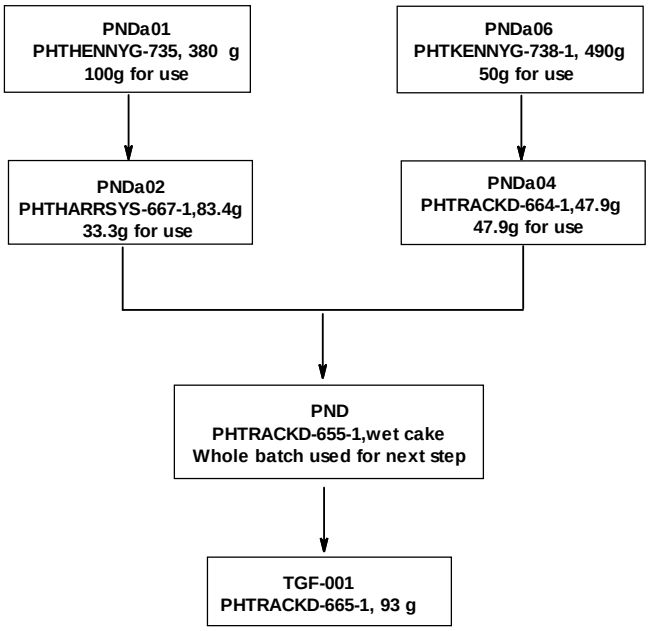
3.1. List of Starting Materials

Name	CAS	Quantity	Purity [%area]
6-methoxypyridin-3-amine	6628-77-9	300g/ bucket	98%
2,4-dichlorobenzoic acid	50-84-0	1000g/Bottle	98%
Na_2CO_3	497-19-8	500 g/Bottle	99%
CuI	7681-65-4	100g/Bottle	99.9%
$POCl_3$	10025-87-3	500mL/Bottle	95%
DIPEA	7087-68-5	500mL/Bottle	99%
Propylene carbonate	108-32-7	6kg/Bottle	97%
Pyrrolidine	123-75-1	1kg/Bottle	98%
Paraformaldehyde	30525-89-4	1kg/ Bottle	96%
2M HCl in EA	7647-01-0	1L/ Bottle	---
H_3PO_4	7664-38-2	500mL/Bottle	85%

3.2. Batch tracking for the synthesis of TGF-001on approx. 100g scale (Phase II)



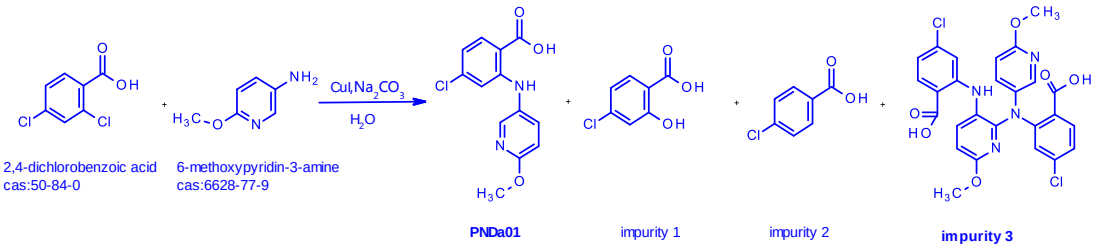
Batch 1



Batch 2

3.3. PNDa01 step

3.3.1. Reaction scheme



3.3.2. Results of PNDa01

- The results of batches made in phase II are shown below. The results consisted with phase 1 work (see ref 10)
- The process of PNDa01 did not change from the phase I procedure doing the second phase of development.
- The assay of PNDa01 is very important (see spec in table 2) for the next step, the HPLC purity has little influence for the next step.

Table 0. Analytical data of PNDa01 5A2MP used in 3 batches.

Batch	Appearance	Water content, %w/w	Purity, %area	Assay, %w/w
2401001	dark brownish color liquid	0.55%	96.18% (HPLC, 254nm)	95.6%
2401002	dark brownish color liquid	0.72%	96.17% (HPLC, 254nm)	95.5%
2401003	dark brownish color liquid	0.56%	96.16% (HPLC, 254nm)	95.0%

Table 1. Results of PNDa01 reaction

No.	6-methoxypyridin-3-amine	2,4-dichlorobenzoic acid (SM1, assay: 99.7%)	Na_2CO_3	CuI	Water	Reaction Temp.	IPC_M1 (16h)	Isolated PNDa01
PHTKENNYG-735	200g (1.0eq) (assay: 95.6%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.48%	Amount: 380.0g Yield: 81.8%
PHTKENNYG-736	200g (1.0eq) (assay: 95.5%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.42%	Amount: 381.0g Yield: 80.6%
PHTKENNYG-737	200g (1.0eq) (assay: 95.0%)	1.4 eq.	2.2 eq.	0.05 eq.	6v/w	95°C	SM2: 2.33%	Amount: 375.0g Yield: 81.4%

3.3.3. The procedure for the preparation of PNDa01 in experiment PHTKENNYG-735

- A suspension of 2,4-dichlorobenzoic acid (435.2g, 2.23 mol, 1.4 eq.), 6-methoxypyridin-3-amine (200g, 1.60 mol, 1.0 eq.), Na_2CO_3 (375.7 g, 3.51 mol, 2.2 eq.) and Copper (I) iodide (15.2 g, 79.7 mmol, 0.05 eq.) in water (1.2L,6v/w) was heated to 95°C and stirred for 16 hrs.
- HPLC showed 6-methoxypyridin-3-amine was 2.48% **(limit: NMT 3.0%)**.

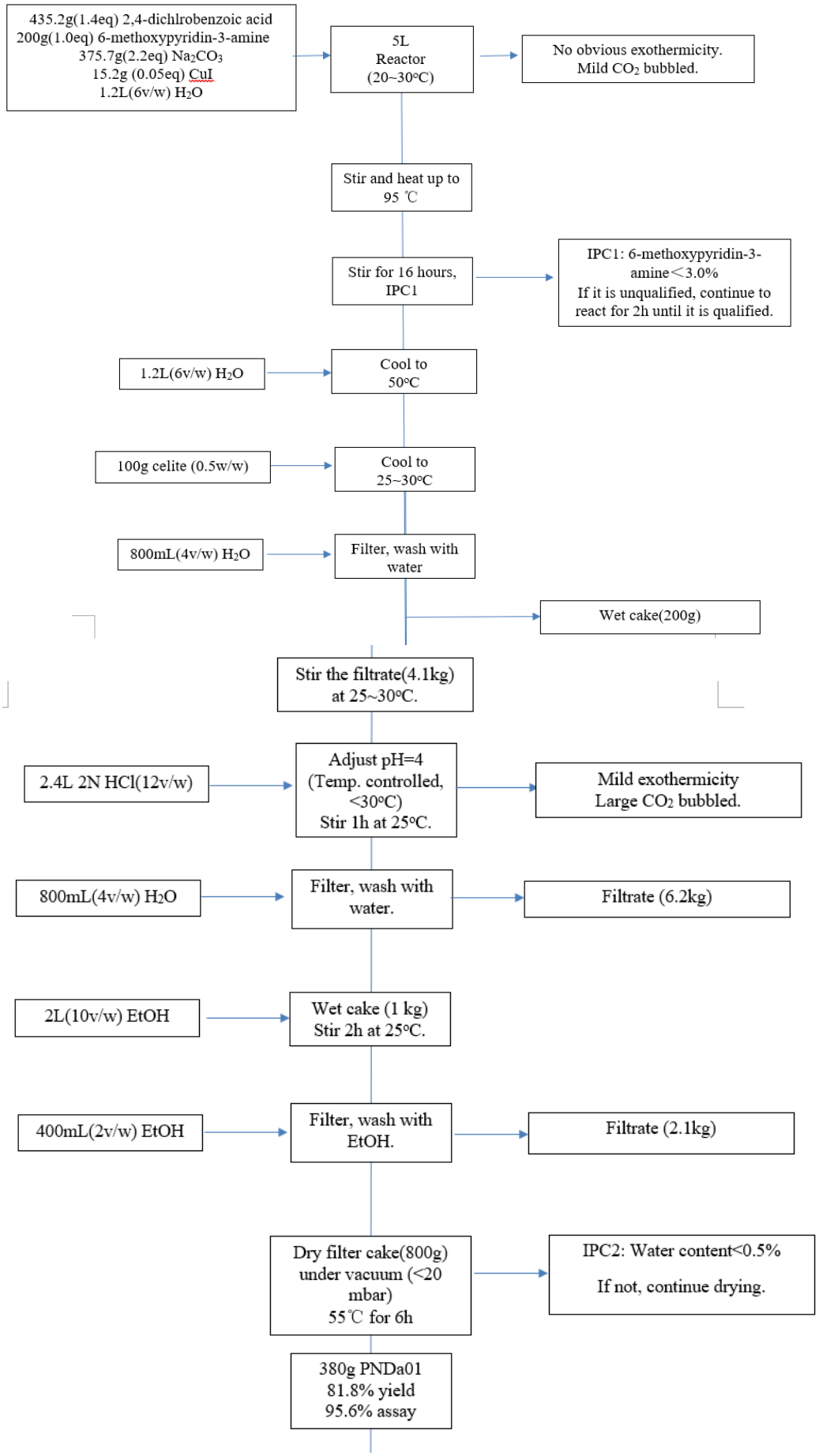
- The reaction solution was added with water (1.2 L, 6v/w).
- The solution was cooled to room temperature(25°C), and charged celite (100g,0.5 w/w) then stirred at 25°C for 0.5 h.
- The suspension was filtered, and the cake was washed with water (400 mL*2,4v/w).
- The combined filtrate was acidified to **pH=4.0** with 2N HCl (approx. 2.4 L, 12v/w, mild exothermicity).
- The suspension was stirred at room temperature (25°C) for 1 h then filtered, and cake was washed with water (400 mL*2,4v/w).
- The crude product was purified by re-slurry in EtOH (2 L,10v/w) at room temperature(25°C) for 2 hrs.
- The suspension was filtered, and cake was washed with EtOH (400 mL,2v/w).
- The collected solid was dried in vacuo at 55°C for 6 h (water: NMT 0.5%w/w) to give 380g PNDa01(Yield 81.8%).

Note: v/w was based on 6-methoxypyridin-3-amine.

Table 2. Analytical data of isolated PNDa01

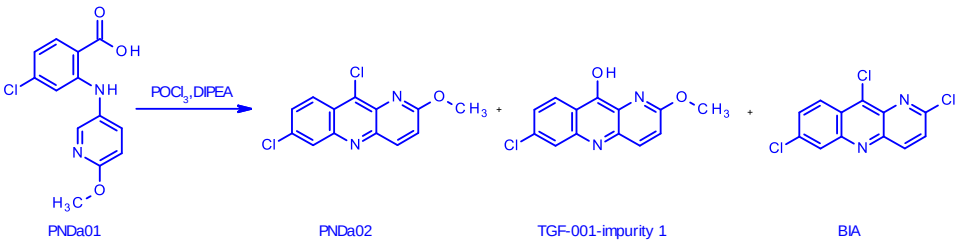
Items	**Specification**	**PHTKENNYG-735**	**PHTKENNYG-736**	**PHTKENNYG-737**
Appearance	Brown solid	Brown solid	Brown solid	Brown solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies	Complies
HPLC purity, %area	NLT 92.0%	93.6%	95.9%	98.1%
HPLC assay, %w/w	NLT92.0%	95.6%	94.1%	96.5%
Water content, %w/w	NMT 0.35%	0.22%	0.09%	0.20%

3.3.4. Flow chart for PNDa01



3.4. PNDa02 step

3.4.1. Reaction scheme



3.4.2. Results of PNDa02

- In phase II development work, it was found that using 15% NaOH aqueous to quench the reaction instead of NH3 H2O gave advantages: (lower production cost, better filtration, no nitrogen-containing wastewater).
- Higher assay is obtained (~95%w/w) compared to the old process(~90%w/w) and the yield was comparable.

Table 3. Results of PNDa02

No.	PNDa01	$POCl_3$	DIPEA	Propylene carbonate	Reaction temperature	IPC_M1 (2h)	Isolated PNDa02
PHTHARRYS-659	Batch PHTKENNYG-735 Assay: 95.6% 100g (1.0eq)	4.0 eq.	4.4 eq.	8v/w	100°C	PNDa01: 0.03% TGF-001 impurity 1:0.5% BIA:0.13% PNDa02:96.2%	Amount: 79.5g Yield: 80.4%
PHTHARRYS-667	Batch PHTKENNYG-735 Assay: 95.6% 100g (1.0eq)	4.0 eq.	4.4 eq.	8v/w	100°C	PNDa01: 0.02% TGF-001-impurity 1:0.16% BIA: 0.22% PNDa02:96.4%	Amount: 83.4g Yield: 82.3%

3.4.3. The procedure for the preparation of PNDa02 in experiment PHTHARRYS-667

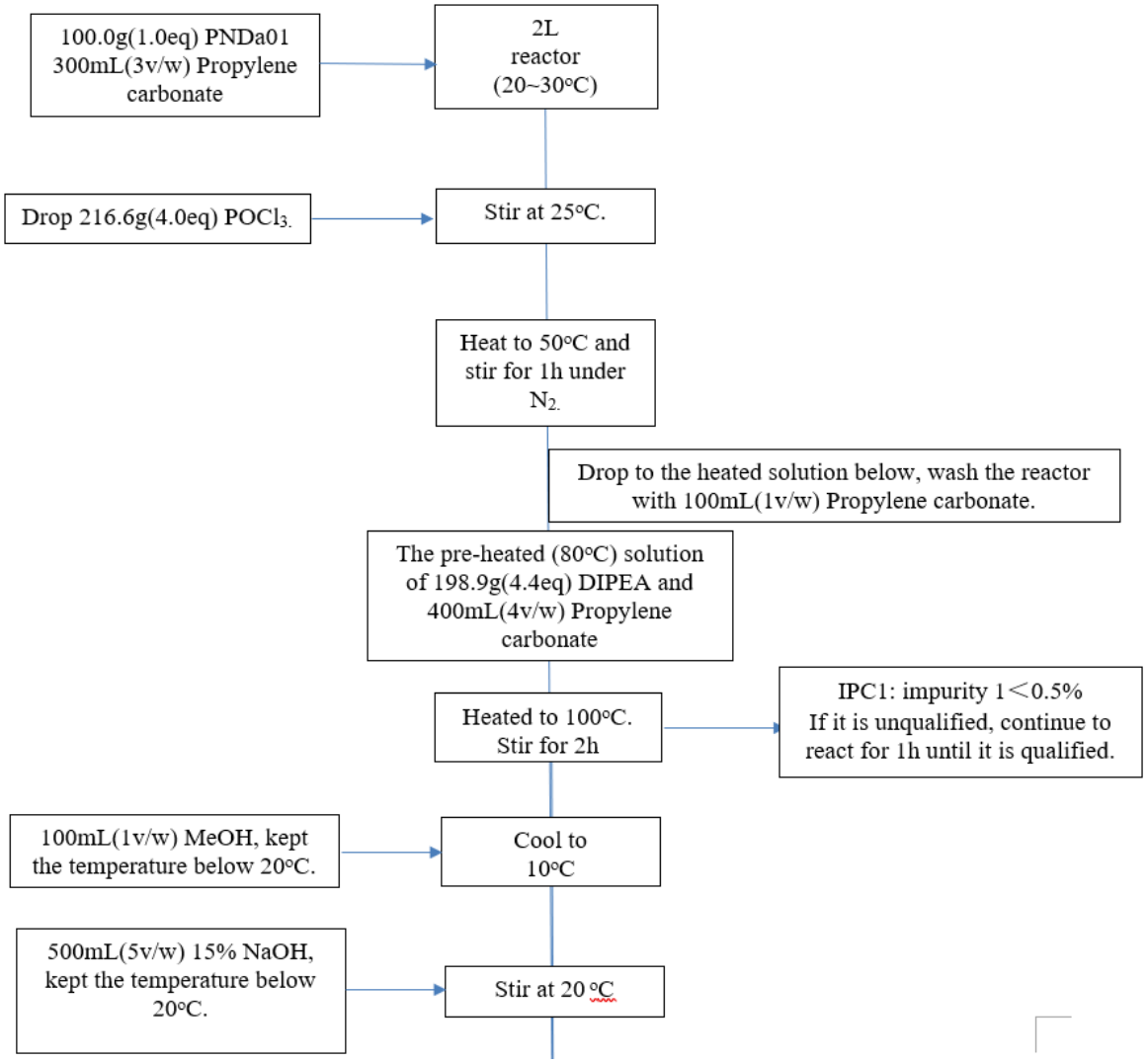
- Charge PNDa01 (100g, 346.2mmol,1.0eq) and Propylene carbonate (300mL, 3v/w) into a 1L flask. $POCl_3$ (216.6g, 1384.8mmol,4.0eq) was then added dropwise into the mixture.
- The mixture was stirred at 50°C for 1h under N_2 atmosphere.
- Charge DIPEA (198.9g, 1523.2mmol, 4.4eq) and Propylene carbonate (400 mL, 4v/w) into another 2L flask and the mixture was heated to 80°C under N_2 atmosphere.
- The prepared acyl chloride was then added dropwise into the mixture (significant increase in temperature during addition: 80°C raised to 87°C).
- After addition, the dropping funnel was washed with Propylene carbonate (100mL, 1V/W).
- The mixture was then reacted at 100°C for 2h. HPLC showed PND01 was 0.03% (**NMT 0.2%**) and TGF-001 impurity1 (PNDa02 intermediate) at RRT 0.75 was 0.16% **(NMT 0.5%)**.
- The mixture was then cooled with an ice bath.

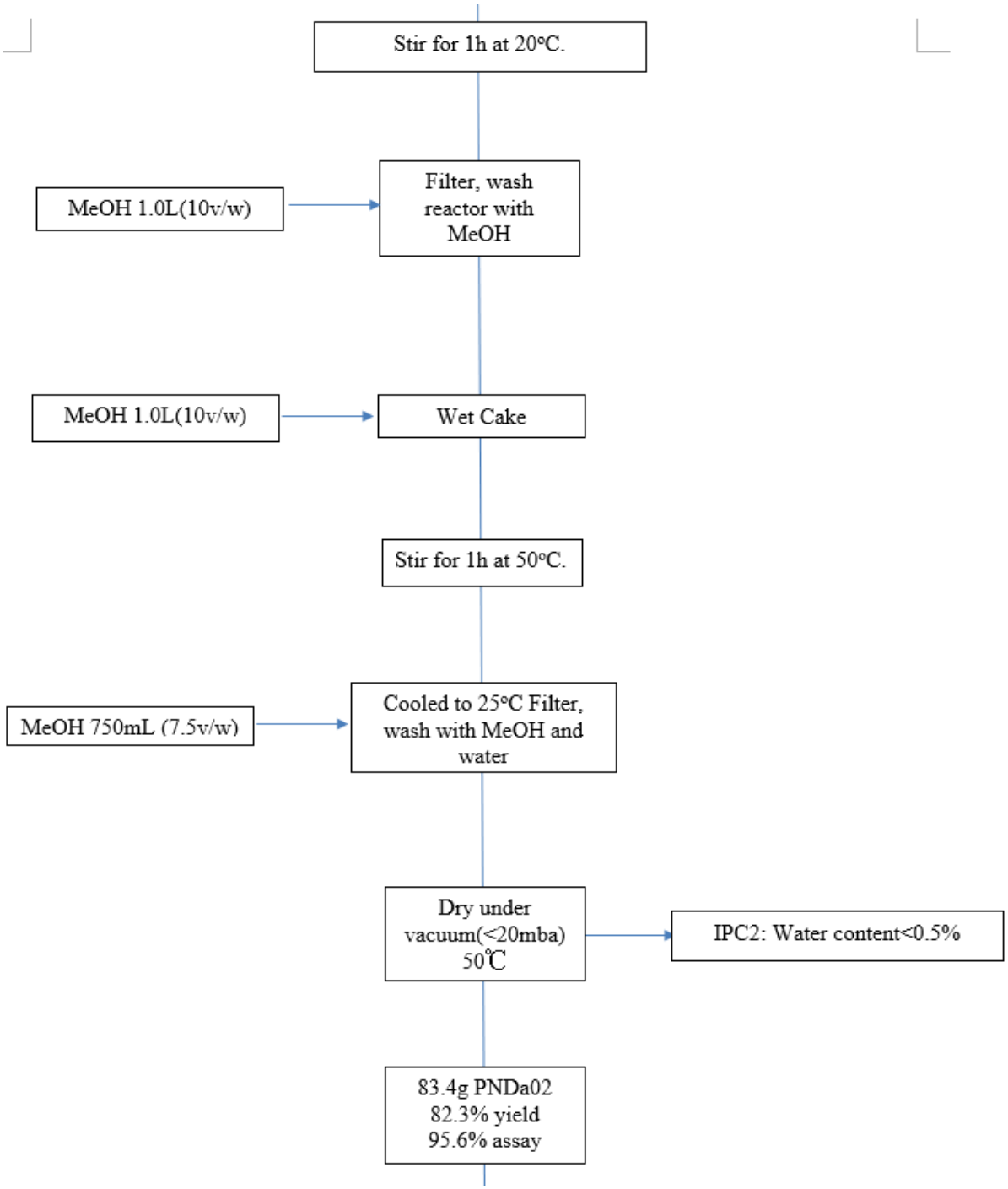
- MeOH (100mL,1v/w) was then added dropwise into mixture and the temperature kept below 20°C. (Significant increase in temperature during addition)
- 500mL 15% NaOH aqueous was then added dropwise into the suspension at ice bath and kept the temperature below 20°C.
- After addition, the mixture was stirred at 20°C for 1h.
- Crude PNDa02 was obtained by filtration.
- The residual PNDa02 on the flask wall was washed with MeOH(1000mL,10v/w).
- MeOH(1000mL,10v/w) was added into the crude PNDa02, and the mixture was stirred at 50°C for 1h.
- The mixture was then filtered under vacuum and the filter cake was washed with (MeOH (750mL,7.5v/w).
- 83.4g PNDa02(Yield:82.3%) as a grey solid was obtained after drying (water: **NMT 0.5%w/w**)

Table 4. Analytical data of isolated PNDa02

Items	**Specification**	**PHTHARRYS-659**	**PHTHARRYS-667**
Appearance	Grey solid	Grey solid	Grey solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies
HPLC purity, %area	NLT 99.0%	99.8%	99.8%
PND01	NMT 0.2%	n. d.	n. d.
BIA	NMT 0.5%	0.13%	0.2%
TGF-001 impurity	NMT 0.5%	n. d.	n. d.
HPLC assay, %w/w	NLT 94.0%	96.3%	95.3%
Water content, %w/w	NMT 0.2%	0.09%	0.08%

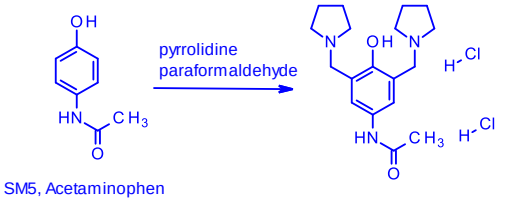
3.4.4. Flow chart for PNDa02





3.5. PNDa06-HCl step

3.5.1. Reaction scheme



SM5, Acetaminophen
CAS:103-90-2
Formula Weight: 151.06

PNDa06-HCl
Formula Weight: 389.16

3.5.2. Results of PNDa06-HCl (using a phase II process showing the following changes)

- Using 2.4eq. Pyrrolidine/paraformaldehyde instead of 2.5eq in PNDa06 reaction.
- Using EtOH(3V) instead of IPA(5V) in salt formation process.
- The results of the modified process were consistent with the phase I work.

Table 5. Results of PNDa06-HCl

No.	Acetaminophen （SM5）	Pyrrolidine/ paraformaldehyde	EtOH	Reaction temperature	IPC by area%(16h)	Isolated PNDa06-HCl
PHTHARRYS-653	200g(1.0eq)	2.4 eq.	5v/w	70°C	SM5:0.07%; PNDa06:99.1%	485.8g (91.3% yield)
PHTKENNYG-738	200g(1.0eq)	2.4 eq.	5 v/w	70°C	SM5:0.02%; PNDa06:99.1%	490.0g (94.5% yield)

3.5.3. The procedure for the preparation of PNDa06-HCl in experiment PHTHARRYS-653

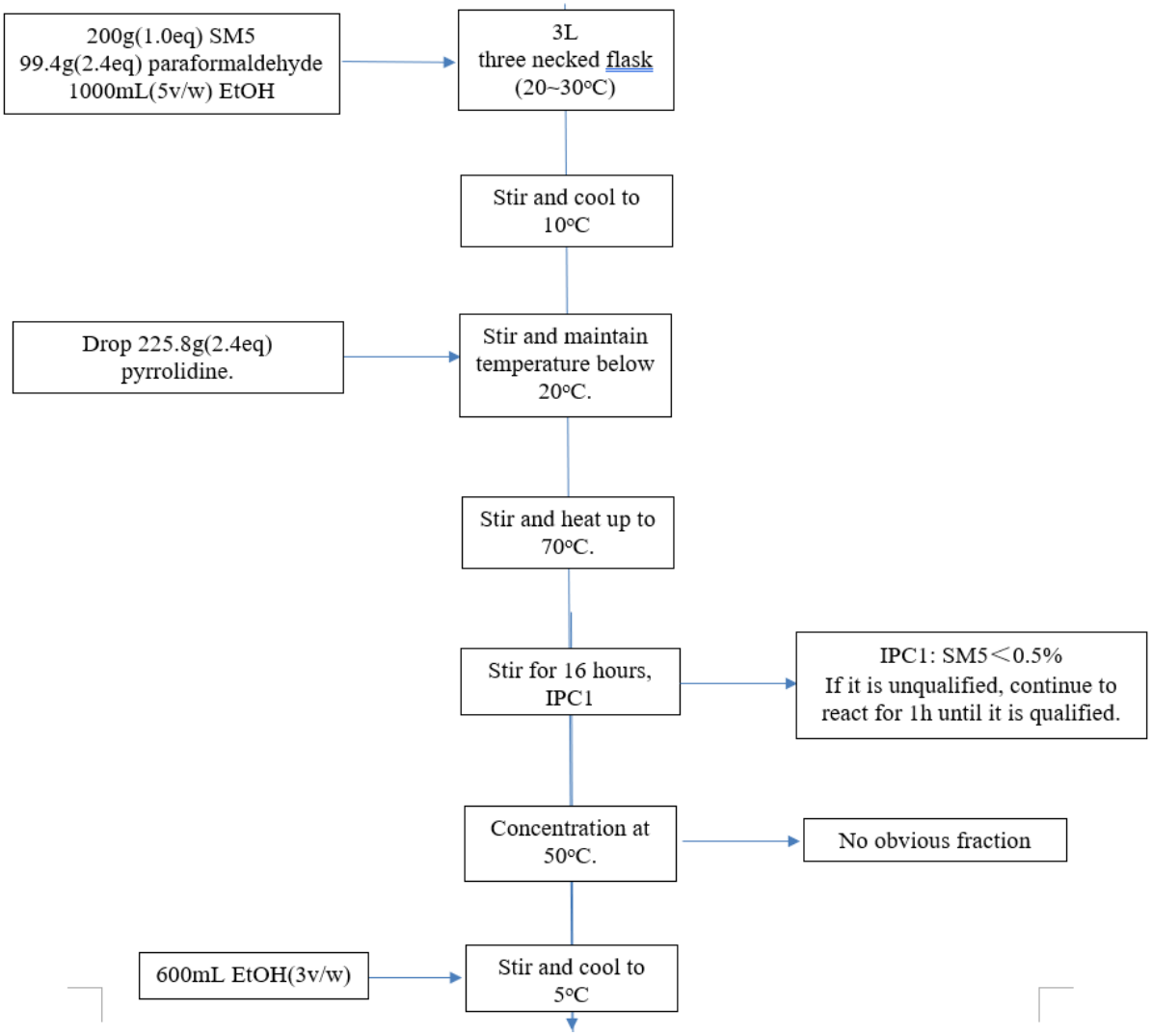
- Charge Acetaminophen (SM5, 200g, 1296.6mmol, 1.0eq), Paraformaldehyde (99.4g, 3111.9mmol, 2.4eq) and Ethanol (1000mL,5v/w) into a 3000mL flask.
- The mixture was then stirred at 30°C for 0.5h.
- Then Pyrrolidine (225.8g, 3111.9mmol, 2.4eq) was added dropwise for 0.5h at 10~20 °C.
- The reaction was raised to 70°C and stirred for 16h under N_2 atmosphere.
- HPLC showed SM5 was 0.07% **(NMT 0.5%)**.
- The solvent was evaporated (50°C) to dryness under reduced pressure to give 455g crude as an orange oil (no obvious fraction).
- EtOH (600mL,3v/w) was added to the residue (orange oil) and the flask was cooled to 5 °C.
- 2M HCl in EA (2400mL,12v/w) was added dropwise to the reaction. EA (1000mL,5v/w) was added to the solution.
- The mixture was heated to 50°C for 10min.
- Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25°C.

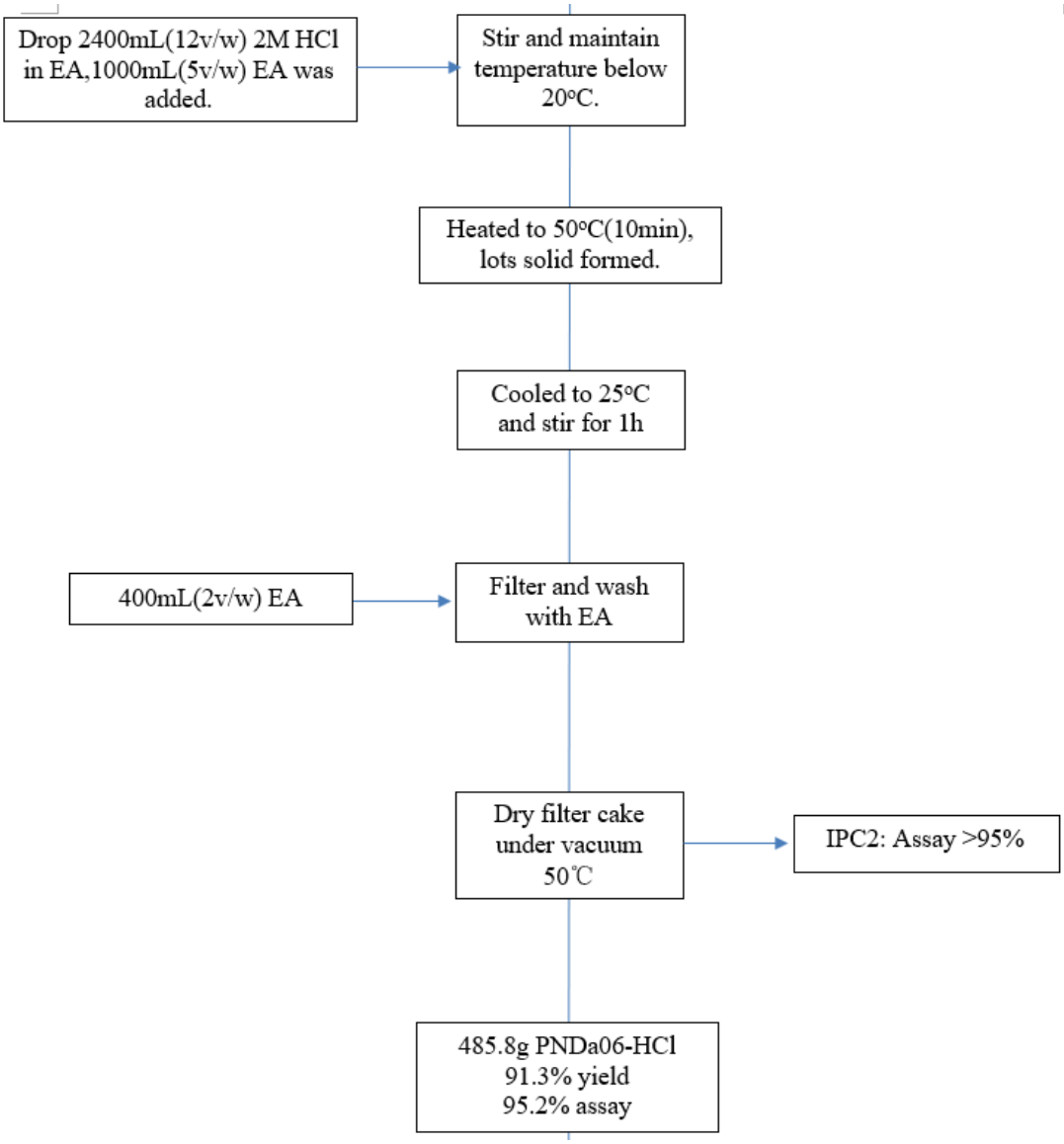
- The solid was filtered and washed with EA (400mL,2v/w).
- The solid was dried under vacuum at 50°C for 3hs to furnish PNDa06-HCl (485.8g, 91.3% yield,95.2% assay).

Table 6. Analytical data of isolated PNDa06-HCl

Items	**Specification**	**PHTHARRYS-653**	**PHTKENNYG-738**
Appearance	Yellow to off-white solid	Yellow to off-white solid	Yellow to off-white solid
Identity by HPLC	Similar retention time for sample and reference solution peak	Complies	Complies
HPLC purity, %area	NLT 98.0%	99.2%	98.3%
Acetaminophen	NMT 0.50%	0.09%	n.d.
HPLC assay, %w/w	NLT 95.0%	95.2%	97.7%

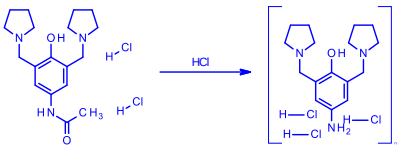
3.5.4. Flow chart for PNDa06-HCl





3.6. PNDa04-HCl step

3.6.1. Reaction scheme



PNDa06-HCl
Formula Weight: 389.16

PNDa04-HCl (not isolated)
Formula Weight: 384.77

3.6.2. Results of PNDa04-HCl

- The results (assay and yield) of 2 batches were consistent with former process in phase I.
- The process has no change in this step.

Table 7. Results of PNDa04-HCl

No.	PNDa06-HCl	HCl aqueous (6mol)	Reaction temperature	IPC_M1 (1 h)	Yield (not isolated)
PHTHARRYS-668	Batch PHTKENNYG-738 Assay: 97.7%, 50g (1.0eq)	4V	100°C	PNDa06: n.d. PNDa04:99.6%	Use for next step as 100% yield
PHTRACKD-664	Batch PHTKENNYG-738 Assay: 97.7%, 50g (1.0eq)	4V	100°C	PNDa06: n.d. PNDa04:99.6%	Use for next step as 100% yield

3.6.3. The procedure for the preparation of PNDa04 in experiment PHTHARRYS-668

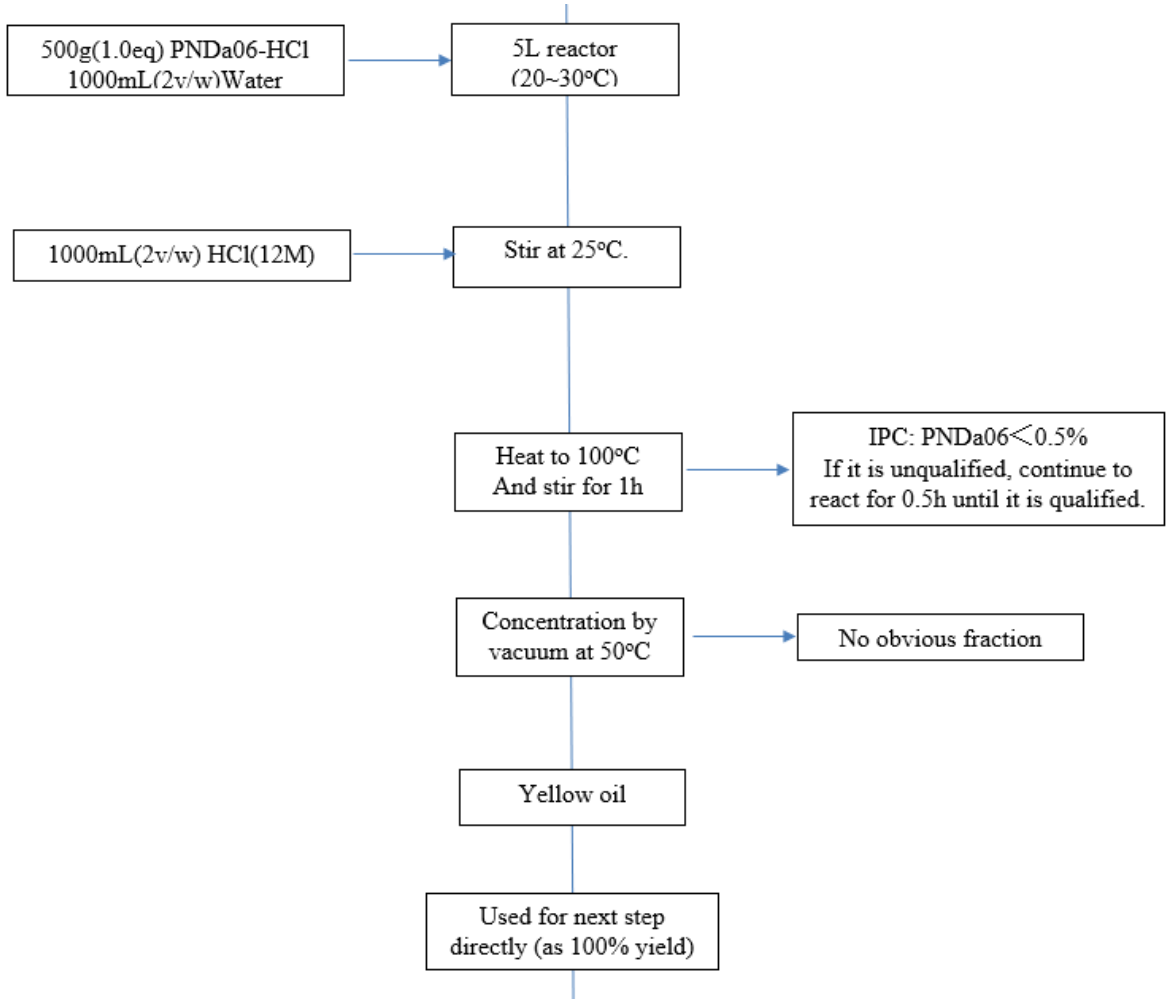
1 Hydrochloric acid (12M/L, 100mL,2v/w) was added to the solution of PNDa06-HCl (50g, 125.1mmol, 1.0eq) in water (100mL).

2 The mixture was stirred at 100°C for 1h.

3 HPLC showed PNDa06=0.0% **(NMT 0.5%)**.

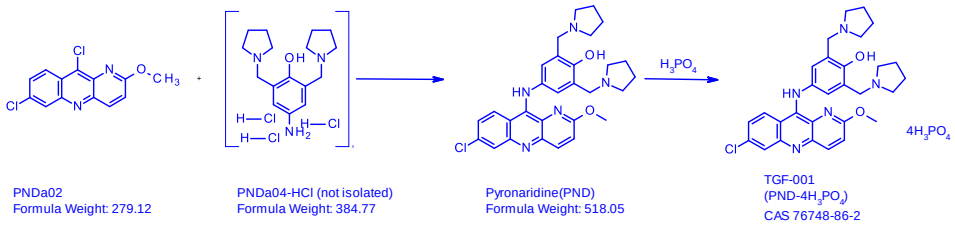
4 The solvent evaporated to dryness (no obvious fraction) at 50°C, the residue was used directly for the next step.

3.6.4. Flow chart for PNDa04-HCl



3.7. PND and TGF-001 steps

3.7.1. Reaction scheme



3.7.2. Results of TGF-001(using a phase II process showing the following changes)

- THF was replaced by acetone in purification step. Acetone can give a stable crystal form. And acetone was easier to dry.
- TGF-001 impurity 1 can be controlled better when reacted at 10℃.
- Residual Cu can meet the specification in modified process. PNDa02 and TGF-001 were easier to filter, maybe the reason.
- The assay of H_3PO_4 was higher in 2 batches. To meet the assay and crystal form requirements need further investigation.

Table 8. Results of TGF-001

No.	PNDa02	PNDa04-HCl	PND IPC (1.5h)	H_3PO_4	Salt formation		Purification	Results
PHTHARRYS-669	PHTHARRYS-667 (Assay: 95.3%w/w) 33.3g (1eq.)	PHTHARRYS-668 (IPC purity:99.6%) (1.1eq)	Batch PHTHARRYS-669 PNDa02:0.10%, PNDa04:0.46% Impurity 1:0.31%, PND:97.7%	6 eq.	Water (5v)	EtOH (10v)	Water(5v)/Acetone (10v) 1eq. H_3PO_4 Wash cake by Acetone	92.4g Yield:86.1%
PHTRACKD-665	PHTHARRYS-667 (Assay: 95.3%w/w) 33.3g (1eq.)	PHTRACKD-664 (IPC purity:99.6%) (1.1eq)	Batch PHTRACKD-665 PNDa02:0.07%, PNDa04:0.53% Impurity 1:0.29%, PND:98.1%	6 eq.	Water (5v)	EtOH (10v)	Water(5v)/Acetone (10v) 1eq. H_3PO_4 Wash cake by Acetone	93g Yield:85.6%

Note: The yield was calculated based on PNDa02.

3.7.3. The procedure for the preparation of TGF-001 in experiment PHTHARRYS-669

- To the solution of PNDa04-HCl (47.9g,124.5mmol,1.1eq) in EtOH (250mL,5v/w) was added PNDa02 (33.3g, 113.7mmol, 1.0eq).
- The suspension was then stirred at 10°C under N_2 atmosphere for 16hs.
- HPLC showed PNDa02=0.10%<0.5%. The solvent was evaporated (50°C) to dryness under reduced pressure to give a semi-solid.
- The solid was dissolved in water(500mL,10v/w). The solution was filtered to remove mechanical impurities. The filtrate was adjusted the pH to 12 with 15% NaOH(70mL).

- Lots of solids precipitated out from the solvent, then continued stirring for 1 hour at 25°C. Collect the solid by filtration, washed with water (250mL,5v/w).
- The solid was transferred to a 1L flask, water (250mL, 5v/w) was added.
- Then H_3PO_4 (85%,78.7g, 682.3mmol, 6.0eq) was added. The mixture was heated to 45°C until a clear solution was found (0.5h).
- EtOH(500mL,10v/w) was added, lots solid was formed.
- The mixture was cooled to 25°C and stirred for 1h. Collect the solid by filtration, washed with 100mL EtOH(2v/w).
- The solid was dried under vacuum at 50°C to furnish TGF-001 (103g).
- Charge TGF-001 crude (103g, 1125.9mmol) into water (500mL,5v/w).
- Then H_3PO_4 (85%,13.1g, 113.7mmol, 1.0eq) was added.
- The suspension was then stirred at 45°C for 0.5h to get a clear solution.
- Acetone (1L,10v/w) was added dropwise. Solid formed as added.
- The mixture was cooled to 25°C and stirred for 1h.
- Collect the solid by filtration, the cake was washed by Acetone (200mL). The cake was dried under vacuum (water: NMT 0.5%w/w, EtOH: NMT 5000ppm, Acetone: NMT 5000ppm) at 50°C to give TGF-001 (92.4g, 86.1% yield for 3 steps).

Table 9. Analytical data for TGF-001 Crude

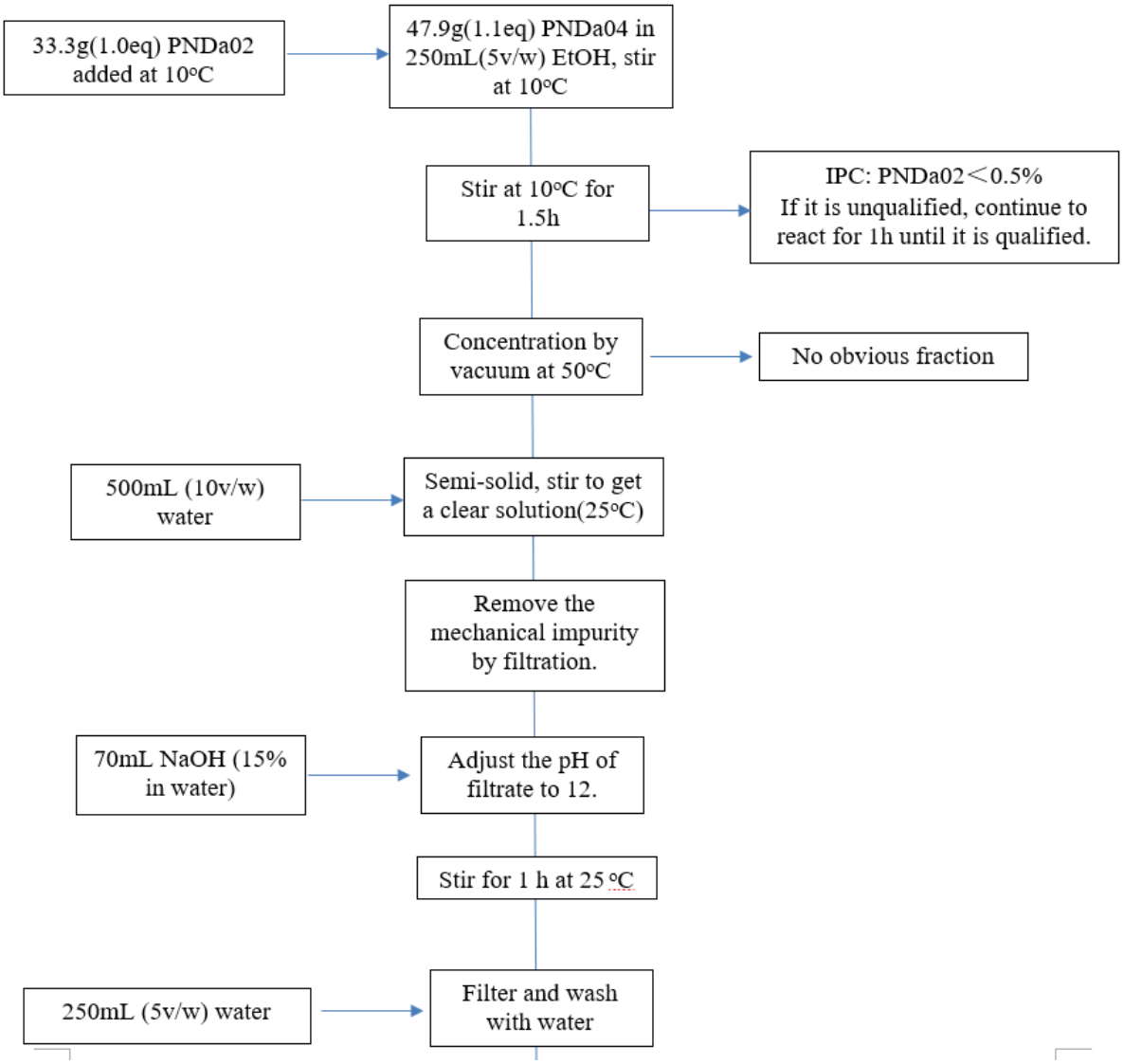
No.	Related substances (HPLC, INV_054926_HPLC_M4)	Residue of EtOH	Water content	Assay (HPLC, INV_054926_HPLC_M4)
PHTHARRYS-669	DIA: 0.11% area DIN: N. D. Impurity 1: 0.16% Total impurities: 0.54% area	0.25% (HNMR)	4.75%(KF)	93.8%
PHTRACKD-665	DIA: 0.07% area DIN: 0.02% area Impurity 1: 0.11% area Total impurities: 0.50% area	1.10% (HNMR)	4.80%(KF)	95.3%

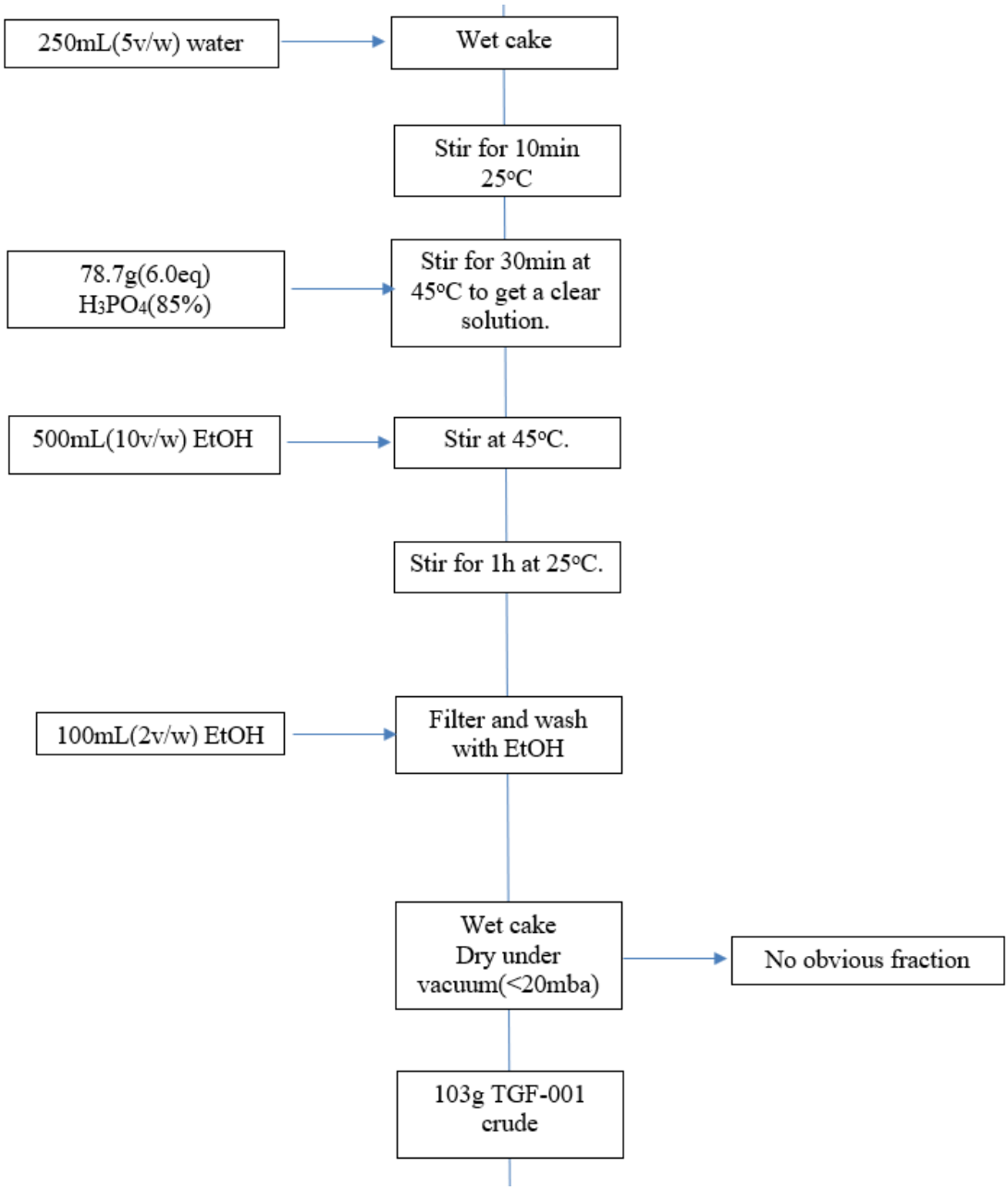
Note: The assay of TGF-001 crude met the specification (exclusive with EtOH and water).

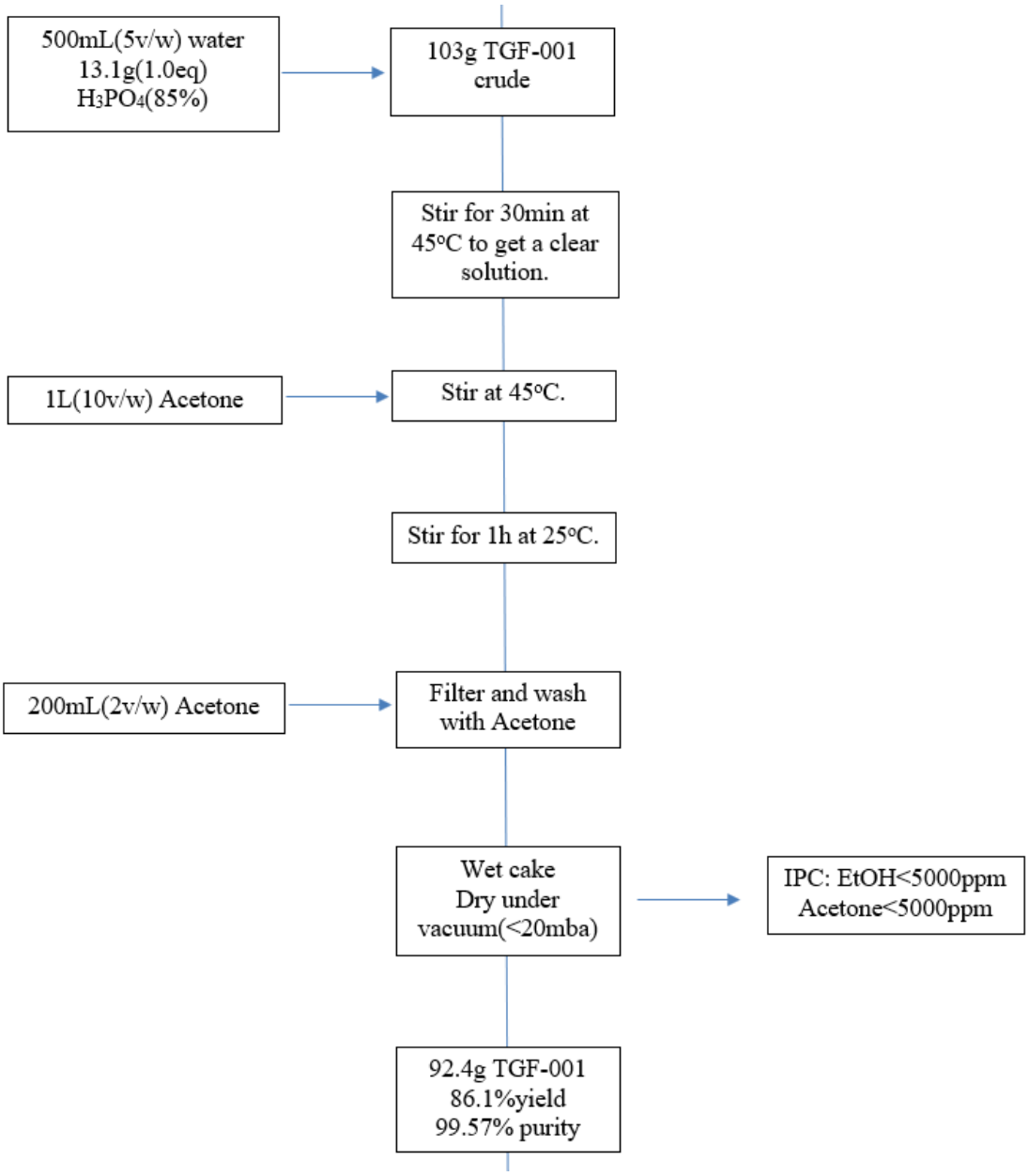
Table 10. Analytical data for final TGF-001

Items	Procedure	PHTHARRYS-669-1	PHTRACKD-665-1
Appearance	Visual inspection	Complies	Complies
Identification	HPLC, INV_054926_HPLC_M4	Complies	Complies
	IR, USP <197>, ATR	Complies	Complies
	Phosphate identification, CP <Malaridine Phosphate>	Positive	Positive
pH value	CP <Malaridine Phosphate>	2.33	2.35
Chloride content	CP <Malaridine Phosphate>	< 0.03%	< 0.03%
Insoluble substances in water	CP <Malaridine Phosphate>	0.2 mg	0.7 mg
Related substances	HPLC, INV_054926_HPLC_M4	DIA: 0.04%w/w DIN: N. D. Impurity 1: 0.02%w/w Total impurities: 0.43% area	DIA: 0.04%w/w DIN: N. D. Impurity 1: 0.02%w/w Total impurities: 0.35% area
Formaldehyde content	CP <Malaridine Phosphate>	< 0.02%	< 0.02%
Pyrrolidine content	CP <Malaridine Phosphate>	Complies	Complies
Loss on drying	CP <Malaridine Phosphate>	0.4%	1.0%
Assay (anhydrous)	HPLC, INV_054926_HPLC_M4	96.1%	95.0%
Residual solvents	INV_054926_GC_M1	225ppm <15 ppm N. D. <15 ppm	217ppm <15 ppm N. D. 132ppm
Elemental impurity (Cu)	ICP-MS, USP <233>	1.8 ppm	2.5 ppm

3.7.4. Flow chart for TGF-001



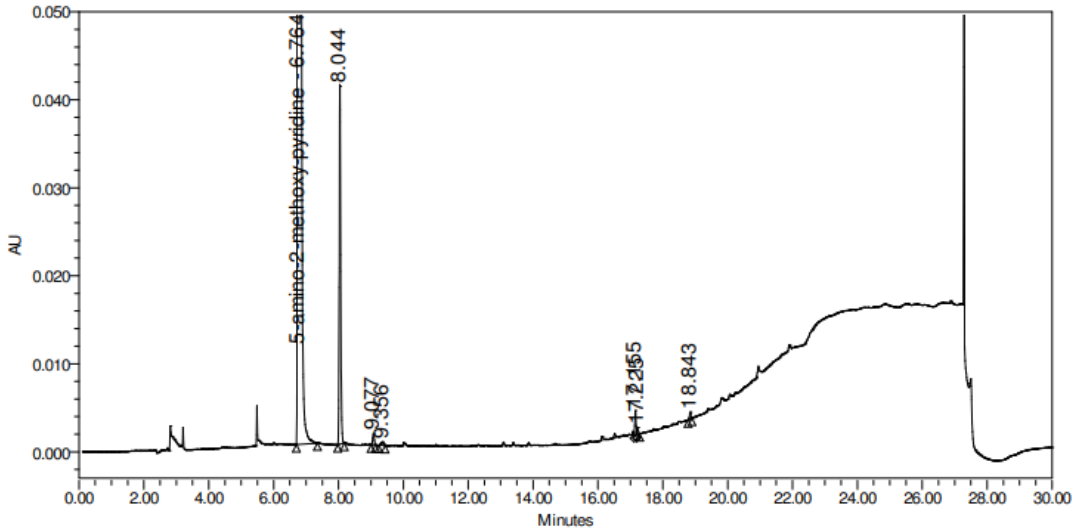




4. Attachments:

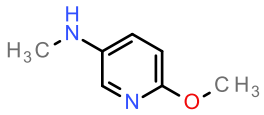
Chromatograms:

Figure 1: HPLC chromatogram of 5A2MP batch 2401001



	Name	RT	RRT	Area	% Area	Resolution
1	5-amino-2-methoxy-pyridine	6.764	1.00	3427998	96.18	
2		8.044	1.19	119314	3.35	
3		9.077	1.34	4096	0.11	
4		9.356	1.38	2259	0.06	
5		17.155	2.54	6433	0.18	
6		17.225	2.55	1812	0.05	
7		18.843	2.79	2053	0.06	

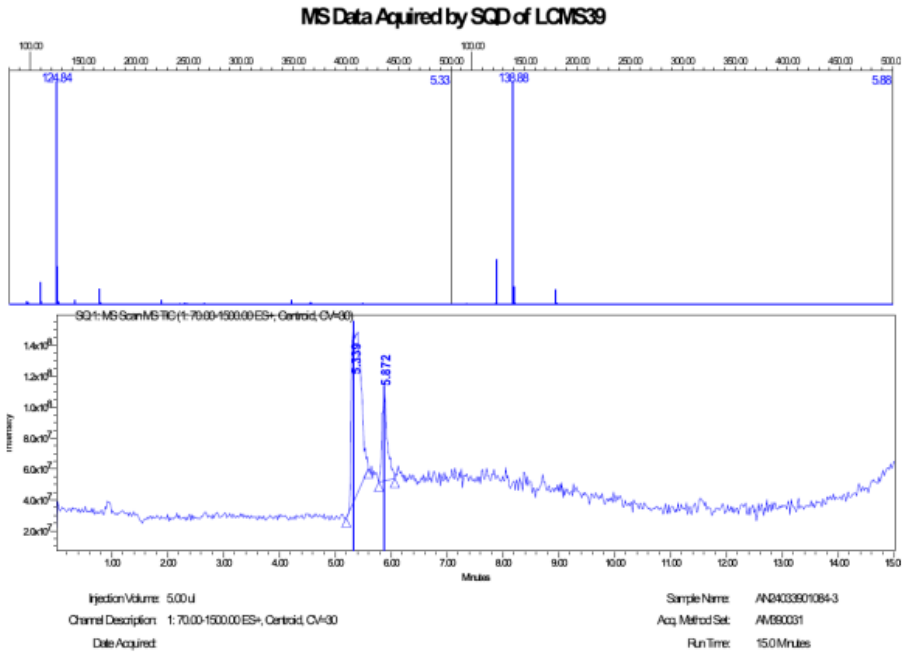
The proposed structure of impurity at RRT=1.19:



Molecular Weight: 138.17
Molecular Formula: $C_7H_{10}N_2O$

The structure of impurity was inferred from LC-MS and ^{1}HNMR.

LC-MS:



[1]HNMR:

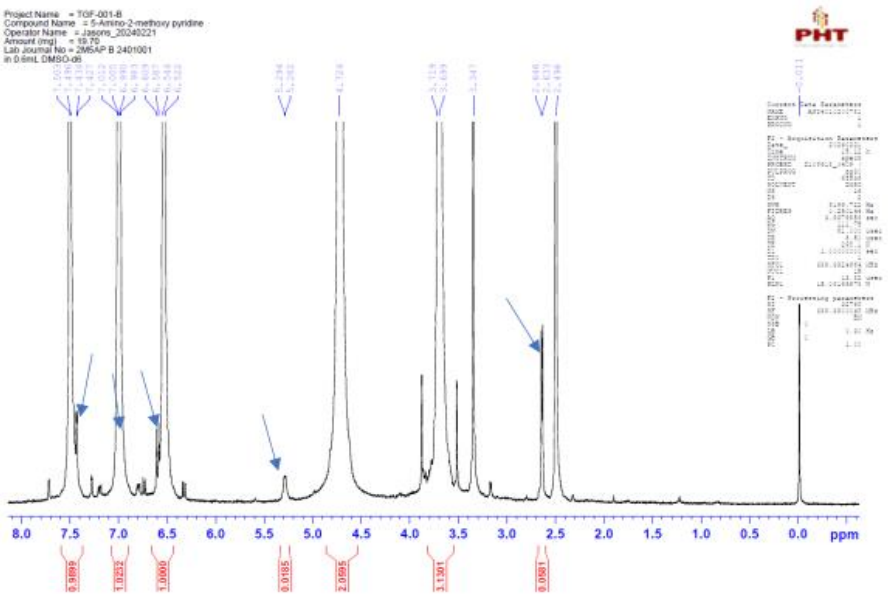
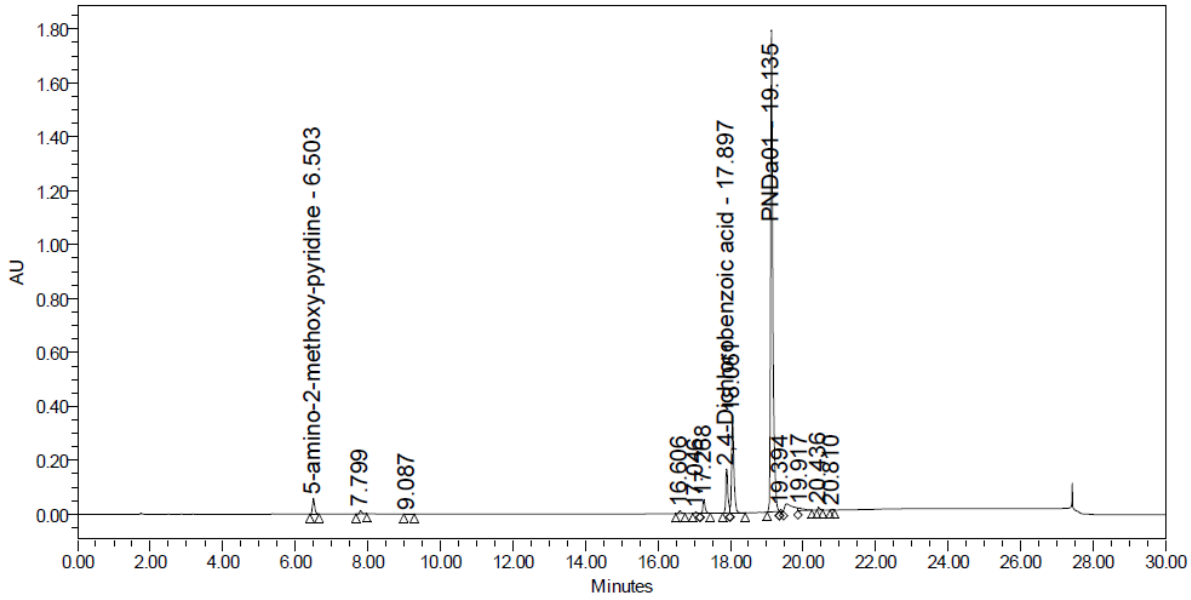


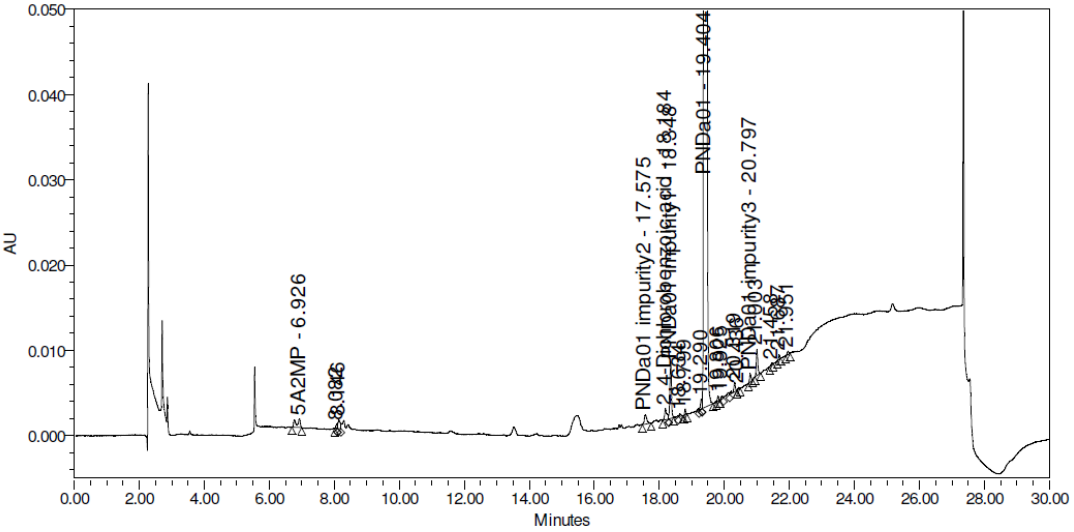
Figure 2: HPLC chromatogram of PNDa01 batch PHTKENNYG-737 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	5-amino-2-methoxy-pyridine	6.503	0.34	242027	2.33	
2		7.799	0.41	52849	0.51	
3		9.087	0.47	3210	0.03	
4		16.606	0.87	44442	0.43	
5		17.046	0.89	20419	0.20	
6		17.268	0.90	220029	2.12	
7	2,4-Dichlorobenzoic acid	17.897	0.94	662185	6.39	
8		18.061	0.94	1543410	14.89	
9	PNDa01	19.135	1.00	7408328	71.45	

	Name	RT	RRT	Area	% Area	Resolution
10		19.394	1.01	29221	0.28	
11		19.917	1.04	97955	0.94	
12		20.436	1.07	34991	0.34	
13		20.810	1.09	9328	0.09	

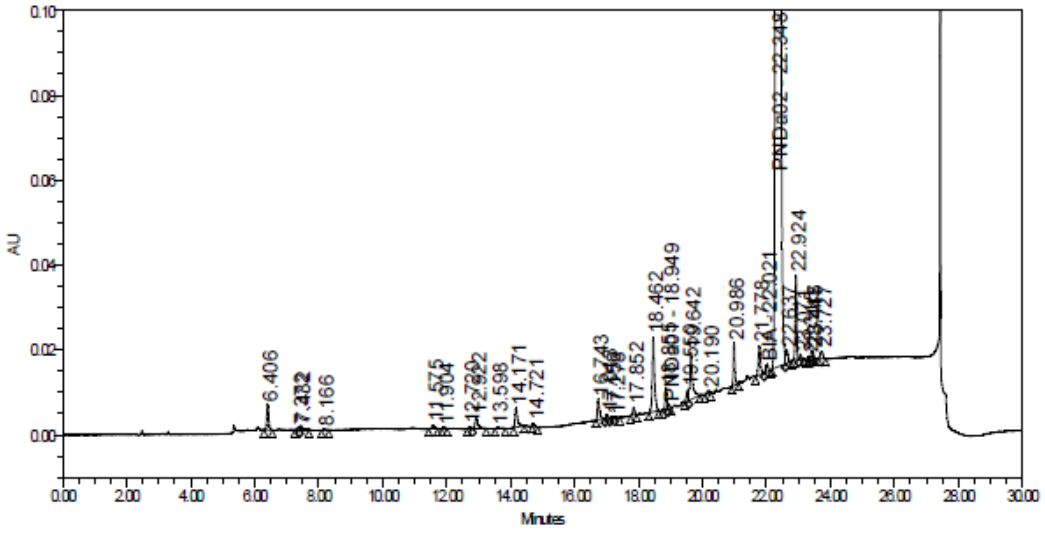
Figure 3: HPLC chromatogram of PNDa01 batch PHTKENNYG-737 (isolated)



	Name	RT	RRT	Area	% Area	Resolution
1	5A2MP	6.926	0.36	8309	0.20	
2		8.082	0.42	1714	0.04	
3		8.146	0.42	4743	0.11	
4	PNDa01 impurity2	17.575	0.91	6056	0.14	
5	2,4-Dichlorobenzoic acid	18.184	0.94	6523	0.15	
6	PNDa01 impurity1	18.348	0.95	21076	0.50	
7		18.634	0.96	1270	0.03	
8		18.799	0.97	1751	0.04	
9		19.290	0.99	3494	0.08	

	Name	RT	RRT	Area	% Area	Resolution
10	PNDa01	19.404	1.00	4157205	98.13	
11		19.806	1.02	1590	0.04	
12		19.925	1.03	1216	0.03	
13		20.319	1.05	4387	0.10	
14		20.436	1.05	693	0.02	
15	PNDa01 impurity3	20.797	1.07	3058	0.07	
16		21.003	1.08	9791	0.23	
17		21.458	1.11	700	0.02	
18		21.687	1.12	1544	0.04	
19		21.951	1.13	1498	0.04	

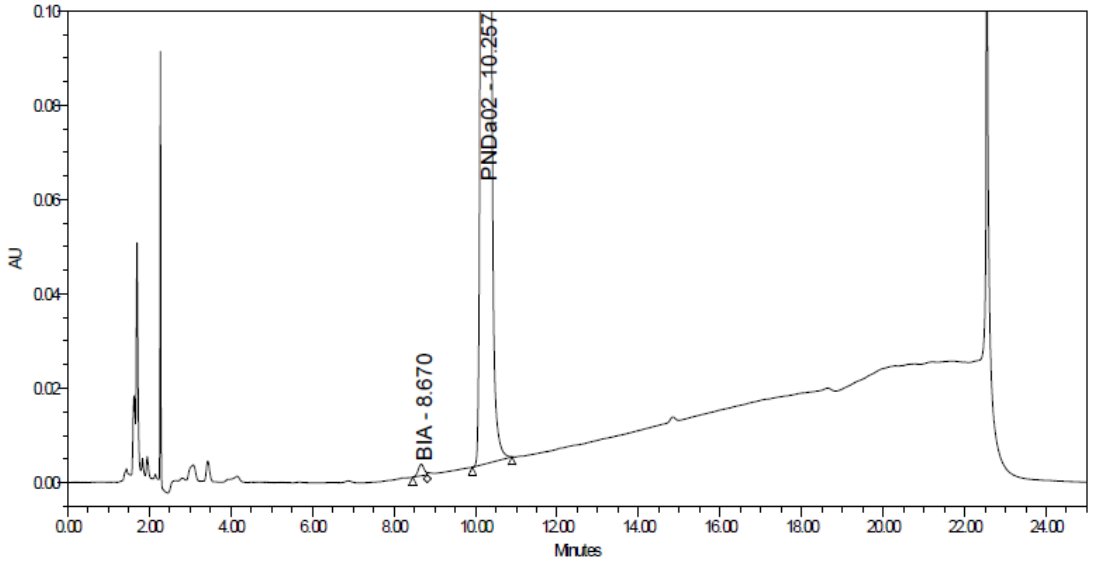
Figure 4: HPLC chromatogram of PNDa02 batch PHTHARRYS-667 (IPC)



	Name	RT	RRT	Area	%Area	Resolution
1		6.406	0.29	24712	0.19	
2		7.372	0.33	2608	0.02	
3		7.432	0.33	6704	0.05	
4		8.166	0.37	1395	0.01	
5		11.575	0.52	5054	0.04	
6		11.904	0.53	1216	0.01	
7		12.720	0.57	2521	0.02	
8		12.922	0.58	15400	0.12	
9		13.598	0.61	1923	0.01	

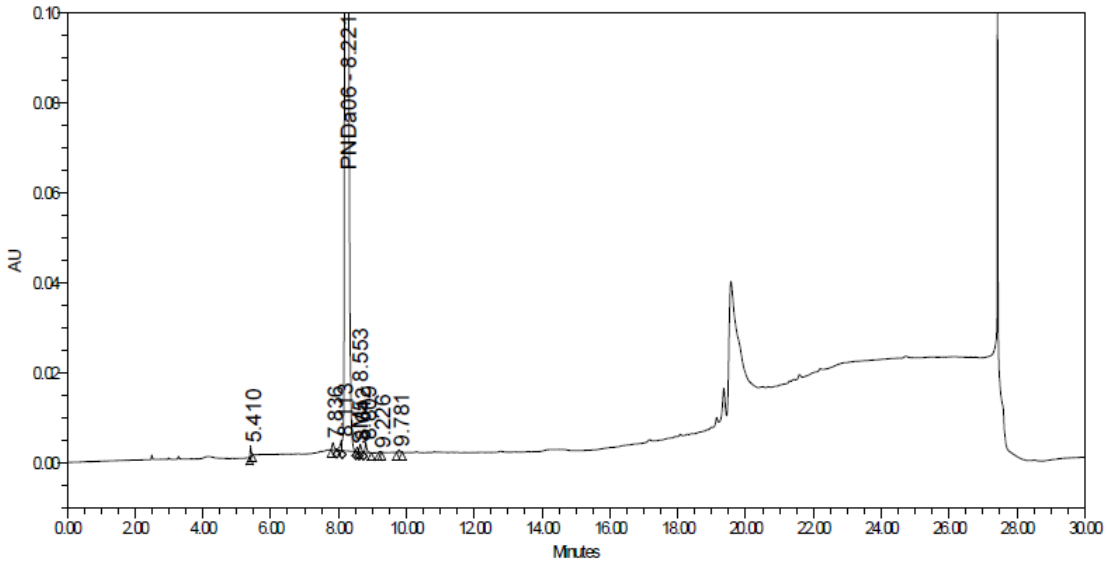
	Name	RT	RRT	Area	%Area	Resolution
10		14.171	0.63	25108	0.19	
11		14.721	0.66	2297	0.02	
12		16.743	0.75	21847	0.16	
13		17.013	0.76	3707	0.03	
14		17.150	0.77	1949	0.01	
15		17.278	0.77	1153	0.01	
16		17.852	0.80	7686	0.06	
17		18.462	0.83	84770	0.64	
18		18.855	0.84	18098	0.14	
19	PNDa01	18.949	0.85	3382	0.03	
20		19.550	0.87	13477	0.10	
21		19.642	0.88	59328	0.45	
22		20.190	0.90	3239	0.02	
23		20.986	0.94	32704	0.25	
24		21.778	0.97	30966	0.23	
25	BIA	22.021	0.99	8842	0.07	
26	PNDa02	22.348	1.00	1282504	96.38	
27		22.637	1.01	14723	0.11	
28		22.924	1.03	59607	0.45	
29		23.071	1.03	3115	0.02	
30		23.315	1.04	1788	0.01	
31		23.415	1.05	7463	0.06	
32		23.447	1.05	4591	0.03	
33		23.727	1.06	9881	0.07	

Figure 5: HPLC chromatogram of PNDa02 batch PHTHARRYS-667 (isolated)



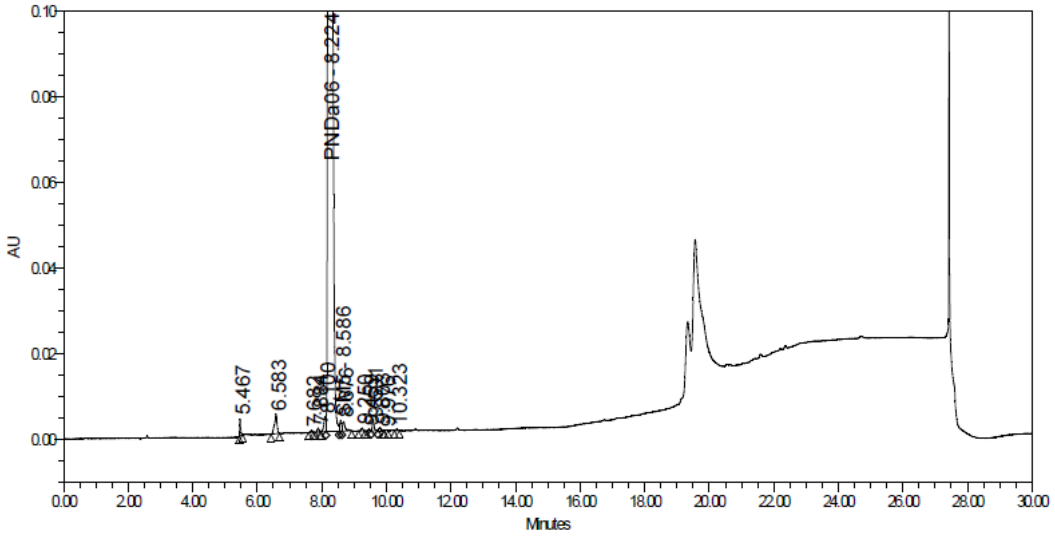
	Name	RT	RRT	Area	%Area	Resolution
1	BIA	8.670		24502	0.20	
2	PNDa02	10.257		11933647	99.80	

Figure 6: HPLC chromatogram of PNDa06-HCl batch PHTHARRYS-653 (IPC)



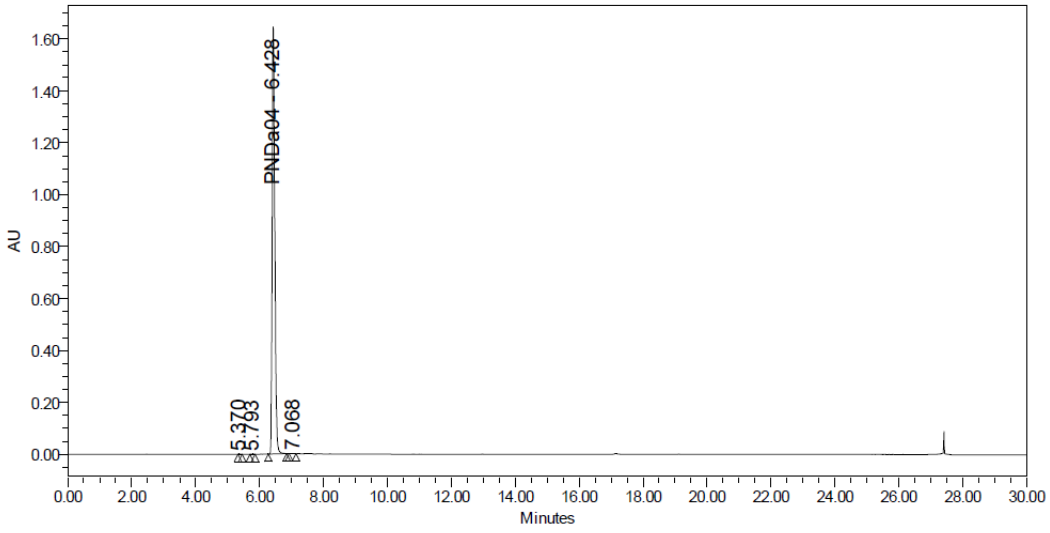
	Name	RT	RRT	Area	%Area	Resolution
1		5.410	0.66	4754	0.10	
2		7.836	0.95	6676	0.14	
3		8.113	0.99	10451	0.22	
4	PNDa06	8.221	1.00	4716758	99.11	
5	SM5	8.553	1.04	3475	0.07	
6		8.642	1.05	6407	0.13	
7		8.809	1.07	7371	0.15	
8		9.226	1.12	844	0.02	
9		9.781	1.19	2263	0.05	

Figure 7: HPLC chromatogram of PNDa06-HCl batch PHTHARRYS-653 (isolated)



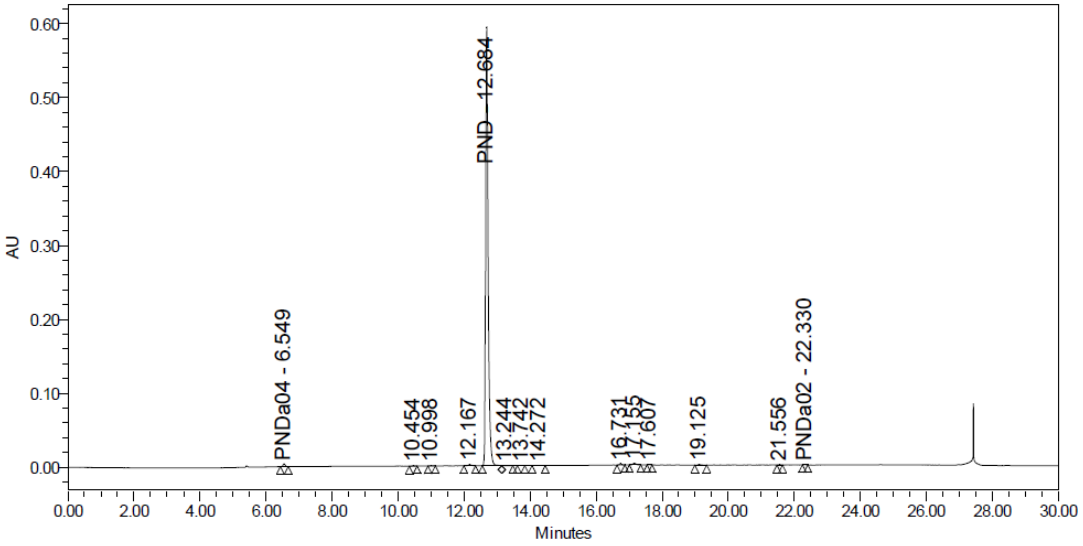
	Name	RT	RRT	Area	%Area	Resolution
1		5.467	0.66	7712	0.07	
2		6.583	0.80	24118	0.21	
3		7.682	0.93	1392	0.01	
4		7.884	0.96	2803	0.02	
5		8.100	0.98	14160	0.13	
6	PNDa06	8.224	1.00	11158602	99.18	
7	SM5	8.586	1.04	10251	0.09	
8		8.676	1.05	12963	0.12	
9		9.250	1.12	2441	0.02	

Figure 8: HPLC chromatogram of PNDa04-HCl batch PHTRACKD-664 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1		5.370	0.84	4933	0.05	
2		5.793	0.90	3128	0.03	
3	PNDa04	6.428	1.00	9436473	99.85	
4		7.068	1.10	6029	0.06	
5	PNDa06	8.200				

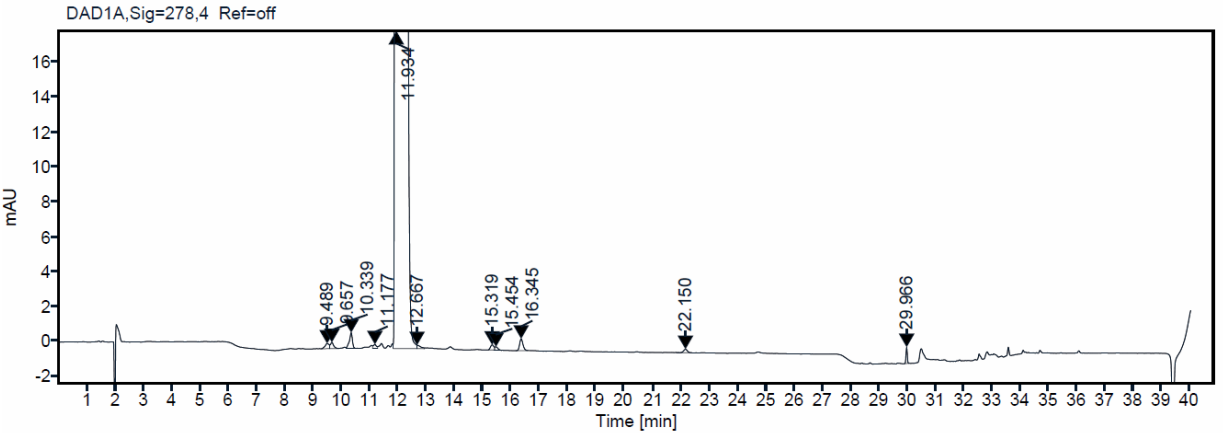
Figure 9: HPLC chromatogram of PND batch PHTRACKD-665 (IPC)



	Name	RT	RRT	Area	% Area	Resolution
1	PNDa04	6.549	0.52	16231	0.51	
2		10.454	0.82	2861	0.09	
3		10.998	0.87	1536	0.05	
4		12.167	0.96	11548	0.36	
5	PND	12.684	1.00	3098797	97.74	
6		13.244	1.04	3544	0.11	
7		13.742	1.08	1030	0.03	
8		14.272	1.13	2832	0.09	
9		16.731	1.32	7963	0.25	

	Name	RT	RRT	Area	% Area	Resolution
10		17.155	1.35	14150	0.45	
11		17.607	1.39	1426	0.04	
12		19.125	1.51	4080	0.13	
13		21.556	1.70	2755	0.09	
14	PNDa02	22.330	1.76	1845	0.06	

Figure 10: HPLC chromatogram of TGF-001(API) batch PHTRACKD-665



Signal: DAD1A,Sig=278,4 Ref=off

Peak Relative Ret Time	Peak Signal To Noise	RT [min]	Width [min]	Area	Height	Area%
		9.489	0.2910	2.8028	0.2891	0.03
		9.657	0.3367	2.9551	0.3562	0.03
		10.339	0.3586	6.6607	0.8962	0.08
		11.177	0.1768	1.7317	0.2234	0.02
		11.934	0.8428	8593.3062	591.2730	99.65
		12.667	0.2703	1.8066	0.1984	0.02
		15.319	0.2257	2.4313	0.2962	0.03
		15.454	0.2265	1.3839	0.1887	0.02
		16.345	0.4425	5.5680	0.6736	0.06
		22.150	0.5554	1.8167	0.1996	0.02
		29.966	0.1587	2.9053	0.9325	0.03
			Sum	**8623.3683**		

Figure 11: XRPD for TGF-001 batch PHTRACKD-665